Supporting Information

Hao and Baltimore 10.1073/pnas.1309990110

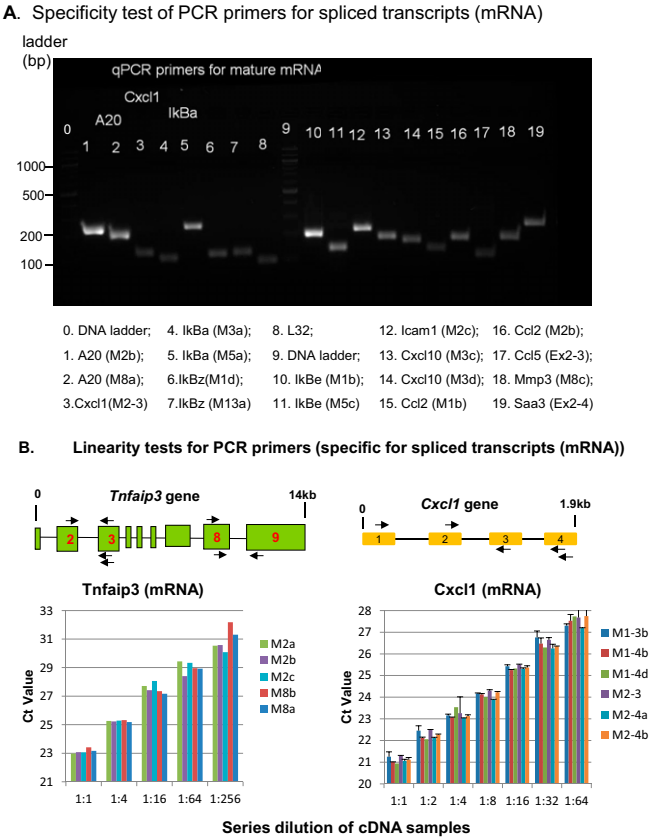

Fig. S1. (Continued)

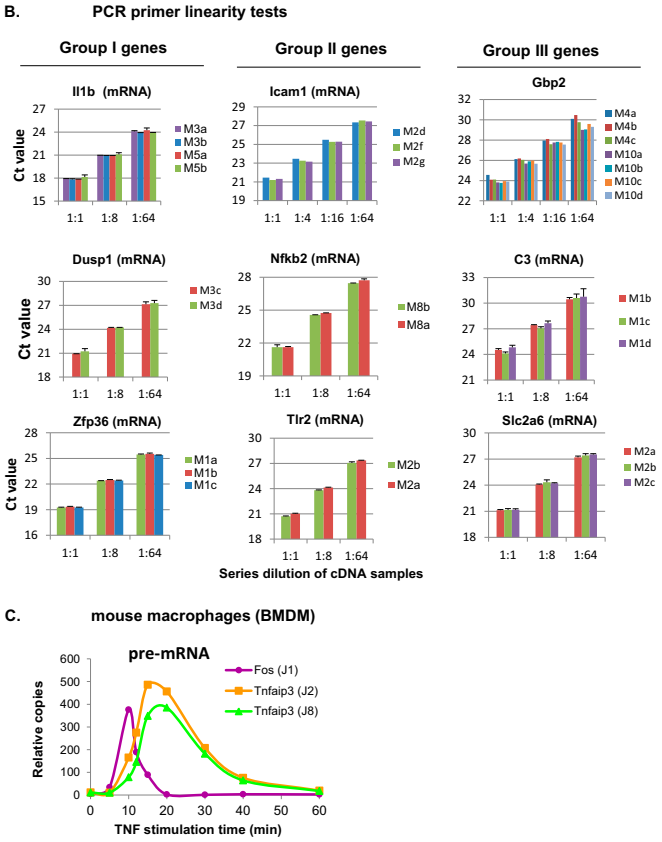

Fig. S1. Related to Fig.1. (*A*) Specific detection of spliced transcripts. An example electrophoresis image of qPCR results using cDNAs from fibroblasts stimulated with TNF for 30 min (for group I and II genes) or 6 h (for group III genes Ccl5, Mmp3, and Saa3). Note that only single bands with sizes corresponding to the transcripts without the intervening intron were amplified with the primers located on two different exons. (*B*) Linearity and comparability test of PCR primers. Spliced transcripts (mRNA) were quantitated by RT-qPCR with different PCR primer pairs using the same cDNA as the template made from a mixture of two samples of cells stimulated with TNF for 15 min and 6 h, respectively. The results are average plus SD of Ct (Cycle number at threshold) values from duplicate samples. (*C*) The pre-mRNA dynamics of Fos and Tnfaip3 detected at indicated exon–intron junctions were monitored in mouse macrophages stimulated with TNF for indicated time using RT-qPCR. The results were average plus SD of duplicate samples and represent at least two independent experiments with similar results. BMDM, bone marrow derived macrophages; C3, complement component 3; Ccl2, chemokine C-C motif ligand 2; Cxcl1, chemokine C-X-C motif ligand 1; Dusp1, dual specificity phosphatase 1; Fos, FBJ murine osteosarcoma viral oncogene homolog; Gbp2, guanylate binding protein 2; IκBa or Nfkbia, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; Icam1, intercellular adhesion molecule 1; Il1b, interleukin 1 beta; L32 or RPL32, ribosomal protein L32; Mmp3, matrix metallopeptidase 3; Nfkb2, nuclear factor of kappa light polypeptide gene enhancer in B-cells 2; qPCR, quantitative PCR; Saa3, serum amyloid A3; Slc2a6, solute carrier family 2, member 6; Tlr2, toll-like receptor 2; *Tnfaip3*, tumor necrosis factor, alpha-induced protein 3; Zfp36, zinc finger protein 36.

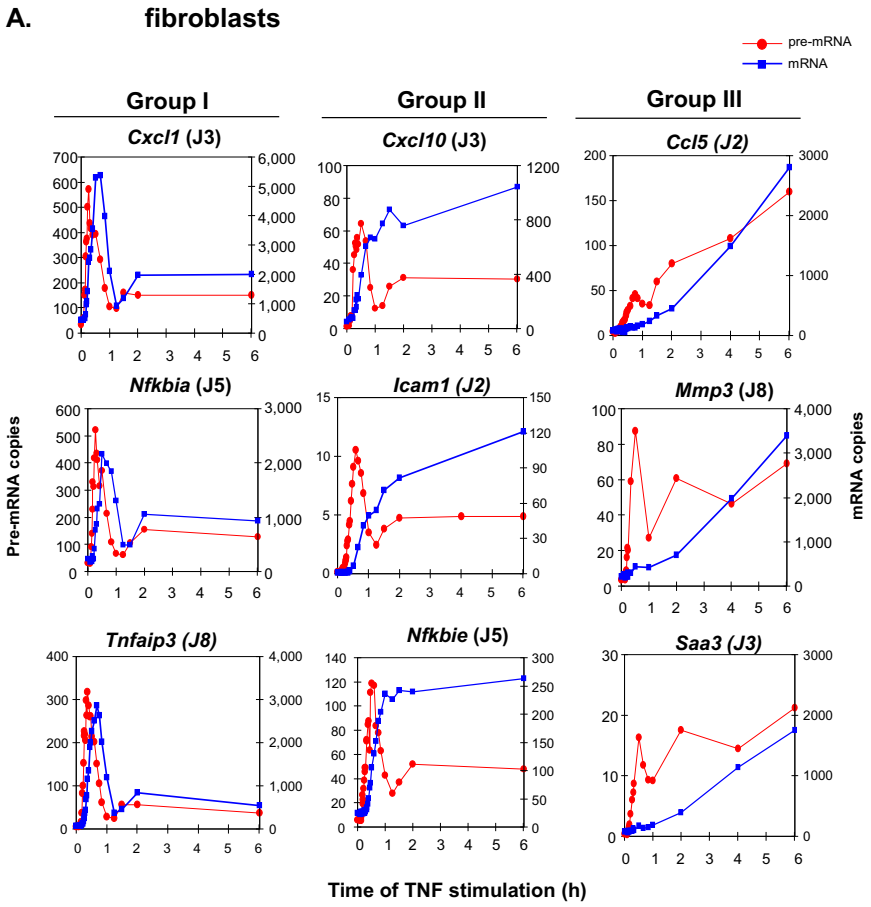

Fig. S2. (Continued)

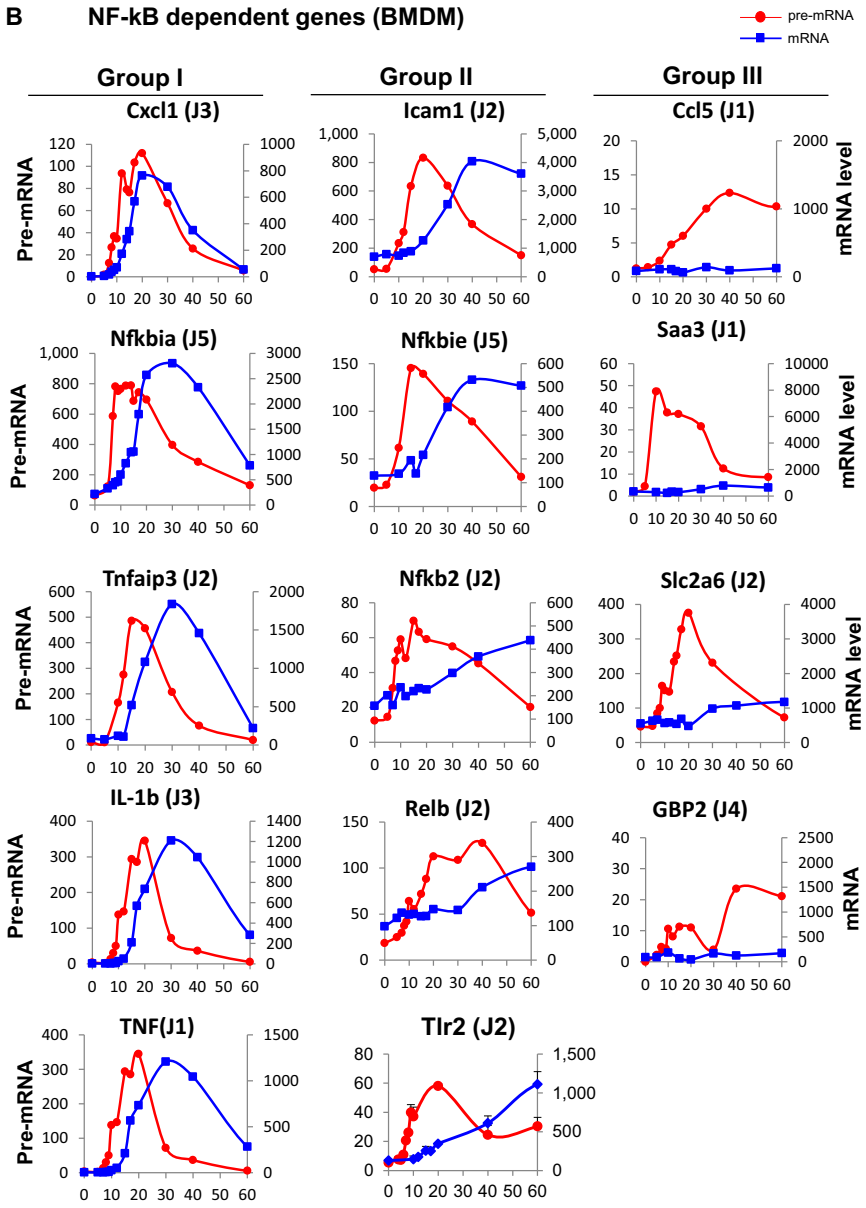

Fig. S2. (Continued)

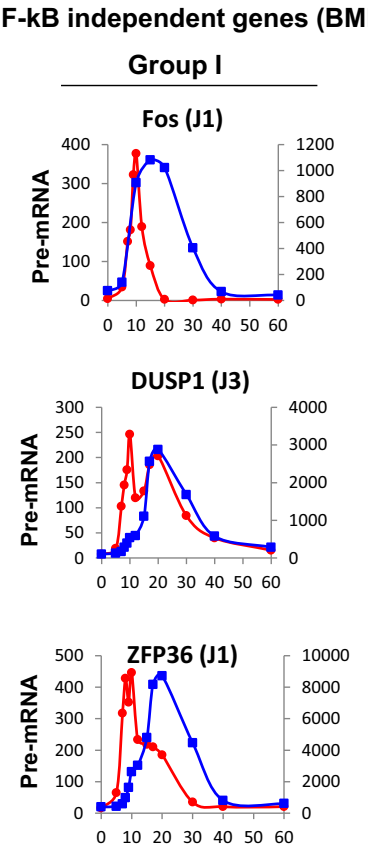

Fig. S2. (Continued)

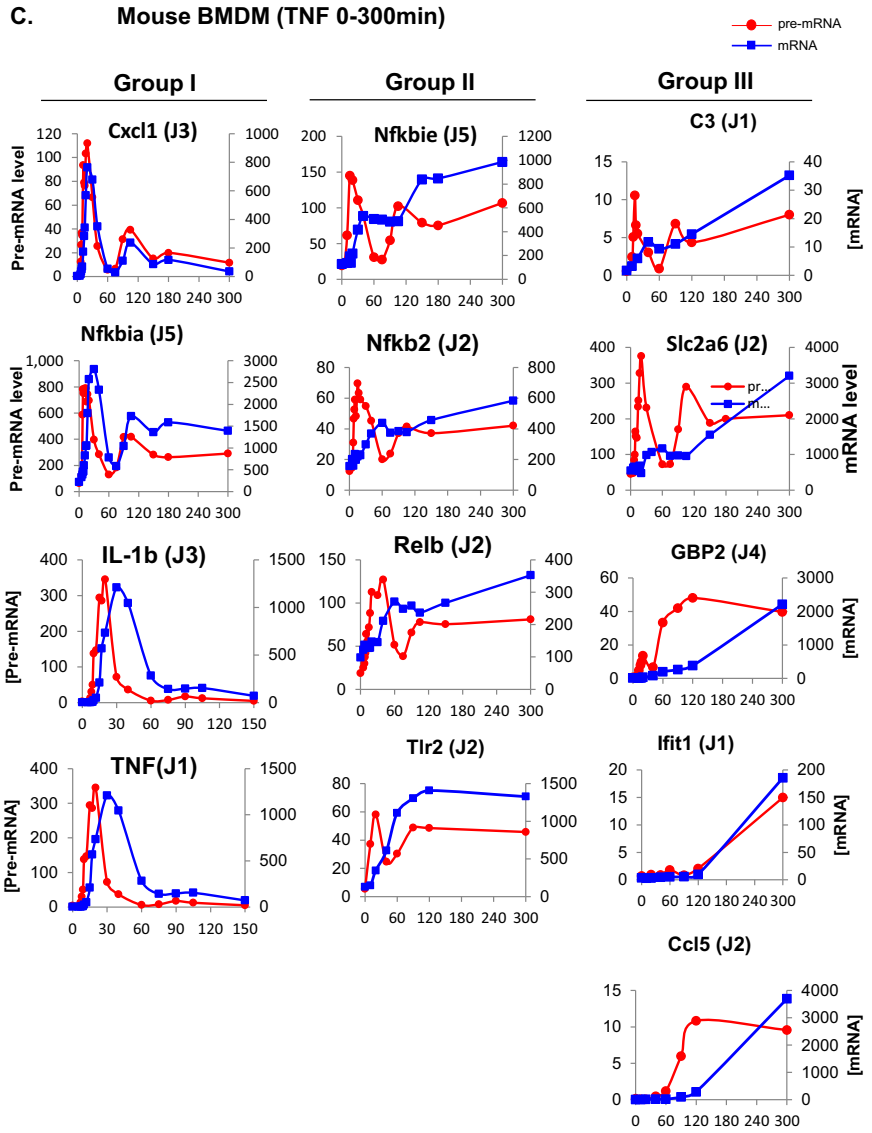

Fig. S2. The dynamic changes of unspliced (pre-mRNA) and spliced (mRNA) transcripts in mouse fibroblasts (*A* showing the same results as in Fig. 2*A* but in a larger time scale) and mouse macrophages (*B* and *C* for larger time scale). The results are the mean plus SD of relative transcript copies from two duplicate samples and represent at least three different experiments with similar results. Ifit1, interferon-induced protein with tetratricopeptide repeats 1; *Nfkbia*, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha; Relb, v-rel reticuloendotheliosis viral oncogene homolog B.

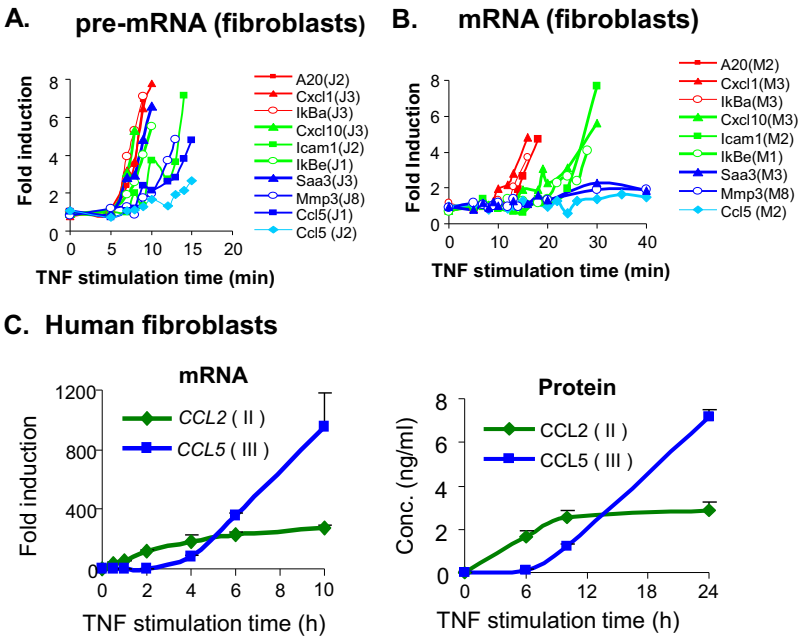

Fig. S3. The temporal order of gene expression is not primarily set at transcription initiation. The changes of the pre-mRNA (*A*) and mRNA (*B*) of genes from group I, II, and III in mouse fibroblasts upon TNF stimulation were measured by RT-qPCR and shown as fold over their basal levels. The results are the average values of duplicate samples with less than 15% variation and represent three independent experiments with similar results. (*C*) The accumulation kinetics of mRNA (*Left*) in the human fibroblasts and protein (*Right*) in the culture medium from the same samples was determined by qPCR and ELISA, respectively. The results are average plus SD of duplicate samples and represent two independent experiments with similar results.

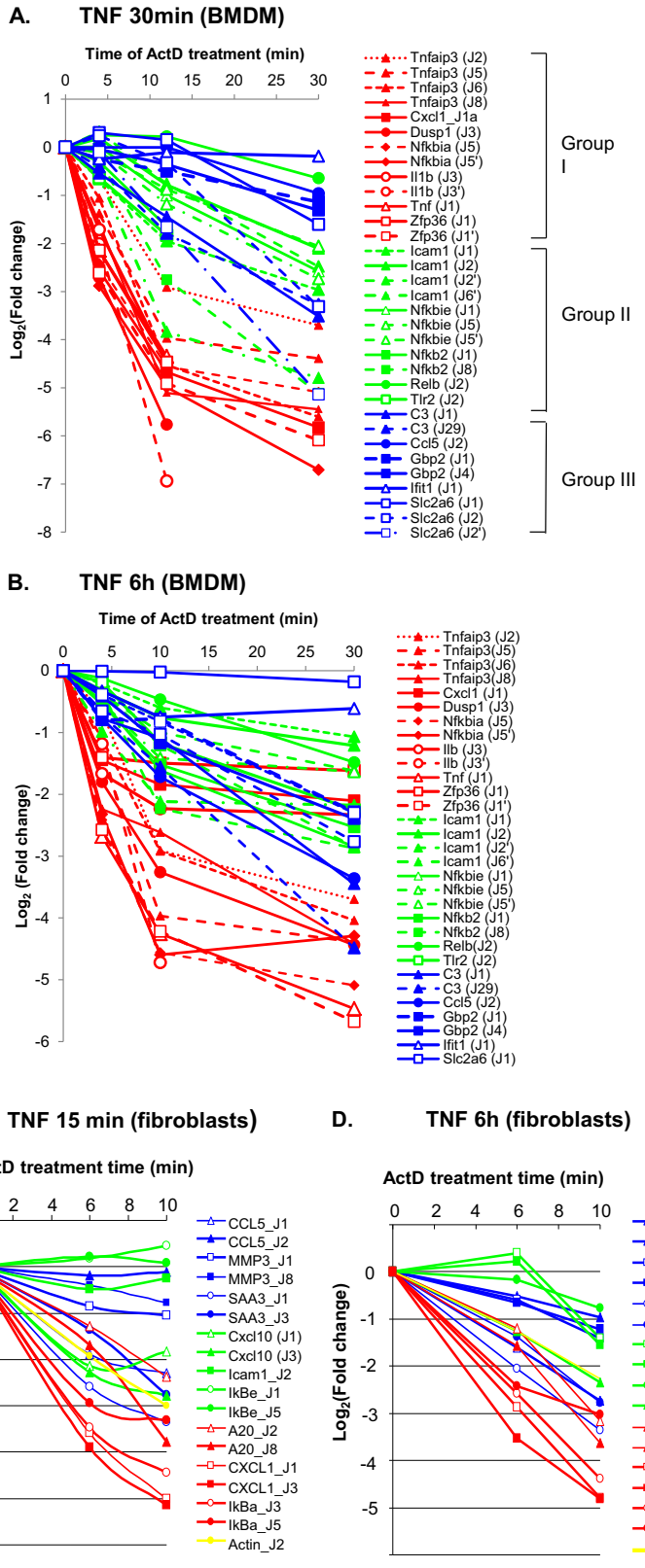

Fig. S4. RNA splicing also regulates the temporal order of gene expression. (*A* and *B*) Mouse macrophages were pretreated with TNF for 30min (*A*) or 6 h (*B*) before addition of Actinomycin D (ActD). (*C* and *D*) Mouse fibroblasts were pretreated with TNF either for 15 min (*C*) or for 6 h (*D*) before addition of ActD for the indicated time. The changes of pre-mRNAs of genes in group I (red), II (green), and III (blue) were monitored by RT-qPCR following ActD addition. The results are the average values of triplicate samples with variation less than 15% and represent two independent experiments with similar results. Dusp1, dual specificity phosphatase 1.

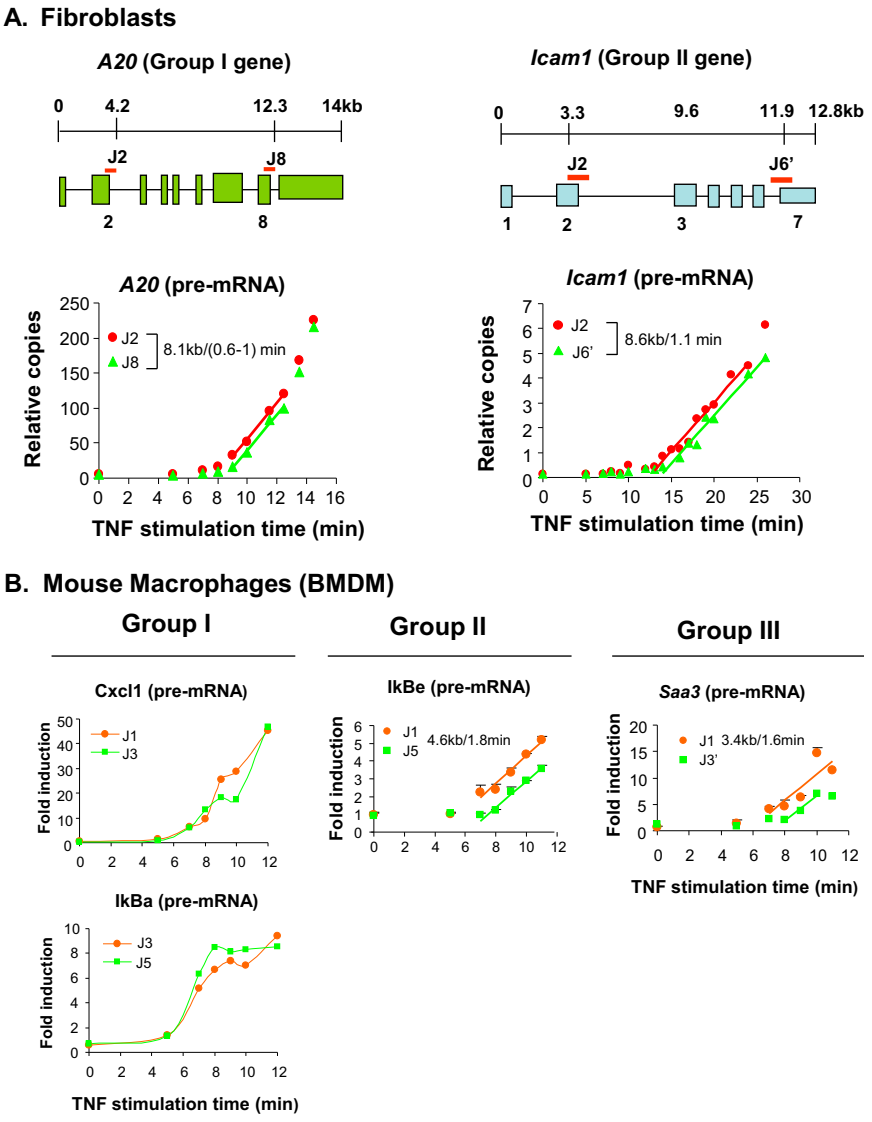

Fig. S5. RNA splicing is slow compared with transcription. (*A*) The scheme of the structures of a group I gene, Tnfaip3 (*A*), and a group II gene, Icam1 (*B*), with the location of two detecting PCR amplicons is shown in *Upper* (not in scale). (*Lower*) The pre-mRNA changes detected at the two indicated regions at indicated time points in mouse fibroblasts were measured by RT-qPCR. The points are an average of duplicate samples with variation less than 15% and represent two independent experiments with similar results. The time lags were estimated between two roughly parallel lines obtained from linear regression analysis of initial rise phase. (*B*) Similar experiments done in mouse macrophages (BMDM). The time lags were estimated between two roughly parallel lines obtained from linear regression analysis of the initial rise phase.

Table S1. Basal expression level of genes in mouse macrophages (two example experiments are shown)

Gene	Subcellular distribution	Function	Basal mRNA	
			Exp. 158	Exp. 168
Group I				
Cxcl1	Secretory	Chemokine	5.9 ± 1	1.62 ± 0.75
Dusp1	Intracellular	Signaling	104 ± 12	135 ± 2.1
FOS	Intracellular	Transcription	75.4 ± 4.5	ND
Il1b	Secretory	Cytokine	3.77 ± 0.89	13.9 ± 2.3
Jun	Intracellular	Transcription	1,071 ± 16	ND
Junb	Intracellular	Transcription	330 ± 1.6	ND
Nfkbia	Intracellular	Transcription	219 ± 95	62.9 ± 4.2
Tnf	Secretory	Cytokine	453 ± 69	61.1 ± 1.1
Tnfaip3	Intracellular	Signaling	86 ± 1.6	27.5 ± 1.8
Zfp36	Intracellular	Rna metabolism	391 ± 44	567 ± 39
Group II				
Cxcl10	Secretory	Chemokine	136 ± 4.6	1.62 ± 0.76
Icam1	Membrane protein	Cell adhesion	704 ± 27	130 ± 11
Nfkb2	Intracellular	Transcription	157 ± 19	104 ± 5.3
Nfkbie	Intracellular	Transcription	110 ± 13	25.6 ± 1.9
Relb	Intracellular	Transcription	98 ± 2.4	41.5 ± 5.7
Tlr2	Membrane protein	Receptor	484 ± 46	151 ± 4.4
Group III				
C3	Secretory	Immunity	96.4 ± 6.8	2.48 ± 0.48
Ccl5	Secretory	Chemokine	86 ± 13	8.56 ± 0.69
Gbp2	Intracellular	Immunity	89 ± 0.81	18.2 ± 1.2
Ifit1	Intracellular	Immunity	121 ± 11	3.35 ± 0.78
Saa3	Secretory	Immunity	351 ± 4.7	7.06 ± 0.41
Slc2a6	Membrane protein	Glucose transporter	548 ± 37	149 ± 38
Housekeeping				
Actb	—	—	ND	25,688 ± 1,678
Rpl32	—	—	4,096	4,096

mRNA level were determined by RT-qPCR and results were normalized by L32 (whose level is set as 2^{12} copies or 4,096). The results were average ± SE of duplicated samples. ND, not determined.

Table S2. Pre-mRNA and mRNA rise time in mouse macrophages in response to TNF stimulation

Gene	Probe	Position (from TSS)	Rise time*, min		Time difference
			Pre-mRNA	mRNA	
Group I					
Cxcl1	J3	1.1 kb	7	10	3
Dusp1	J3	1.9 kb	6	9	3
Il1b	J3	3.0 kb	6	10	4
Nfkbia	J5	2.2 kb	6	8	2
Tnf	J1	0.4 kb	5	8	3
Tnfaip3	J2	4.2 kb	7	12	5
Zfp36	J1 (3′)	0.7 kb	6	9	3
Group II					
Cxcl10	J3	1.1 kb	8	12	4
Icam1	J2	3.2 kb	9	15	6
Nfkbie	J1	0.6 kb	7	15	8
Nfkb2	J2	1.2 kb	7	17	10
Relb	J2	1.5 kb	7	~40	~30
Tlr2	J2	0.6 kb	7	15	8
Group III					
Ccl5	J1	0.2 kb	9	~40	>30
Gbp2	J1	0.2 kb	7	~40	>30
Ifit1	J1	0.2 kb	9	~40	>30
Saa3	J3 (3′)	3.1 kb	7	~40	>30
Slc2a6	J2 (3′)	1.8 kb	7	~40	>30

*The rise time is defined by the point after which the values are significantly higher than basal level ($P < 0.05$ by t test). The PCR primers are located across the 5′ end of the indicated intron or unless it is specially indicated.

Table S3. Half-life of pre-mRNA in mouse bone marrow derived macrophages

Group	Gene	Total intron no.	Average intron size (nt)	Analyzed intron	Size (nt)	Half-life (min)	
						TNF (15 min)	TNF (6 h)
Group I							
	Cxcl1	3	307	In 1	89	1.6 ± 0.3	2.8 ± 0.9
	Cxcl1	3	307	In 3	718	1.3 ± 0.6	-
	Dusp1	3	313	In3	334	1.5 ± 0.4	1.8 ± 0.4
	Nfkbia	5	332	In 3	260	1.2 ± 0.2	1.4 ± 0.2
	Nfkbia	5	332	In 5	436	1.7 ± 0.2	1.9 ± 0.3
	Il1b	6	869	In3	1544	2.3 ± 0.5	2.1 ± 0.4
	Tnf	3	329	In1	516	1.9 ± 0.4	1.4 ± 0.2
	Tnf	3	329	In3	294	1.5 ± 0.2	1.4 ± 0.2
	Tnfaip3	8	1259	In 2	3145	4.0 ± 0.2	4.6 ± 2.7
	Tnfaip3	8	1259	In5	1131	2.9 ± 1.2	3.1 ± 0.9
	Tnfaip3	8	1259	In 6	219	3.8 ± 1.7	3.6 ± 1.0
	Tnfaip3	8	1259	In 8	560	2.5 ± 0.9	1.8 ± 0.1
	Zfp36	1	680	In1	680	1.5 ± 0.3	1.6 ± 0.5
Group II							
	Cxcl10	3	383	In 2	194	2.2 ± 0.1	1.8 ± 0.4
	Cxcl10	3	383	In 3	419	2.7 ± 0.2	1.6 ± 0.2
	Icam1	6	1716	In 1	2817	9.2±1.1	21±4
	Icam1	6	1716	In 2	6364	9.7 ± 2.3	10 ± 2
	Icam1	6	1716	In 6 (3′)	90	5.2 ± 0.8	4.6 ± 0.4
	Nfkbie	5	1006	In 1	2180	9.5 ± 0.8	4.9 ± 1.5
	Nfkbie	5	1006	In 5	1354	10.8 ± 1.9	6.9 ± 1.8
	Nfkb2	22	195	In1	1109	6.8 ± 0.5	6.1 ± 0.6
	Nfkb2	22	195	In8	237	5.3 ± 0.4	7.1 ± 1.1
	Relb	11	1910	In8	1060	38 ± 6	19 ± 2
	Tlr2	2	1241	In2	2099	6.2 ± 0.6	6.7 ± 1.3
Group III							
	C3	40	476	In1	1073	6.7 ± 0.4	6.9 ± 0.8
	C3	40	476	In29	1248	4.5 ± 0.6	4.4 ± 0.7
	Ccl5	2	2104	In 1	1118	-	2.0 ± 0.5
	Ccl5	2	2104	In 2	3091	-	5.0 ± 0.8
	Gbp2	10	1489	In1	2861	19 ± 3	11 ± 2
	Gbp2	10	1489	In4	4346	14 ± 3	8 ± 2
	Ifit1	1	6483	In1	6483	-	17 ± 8
	Slc2a6	9	500	In1	513	13 ± 2	13 ± 1
	Slc2a6	9	500	In2	1033	8 ± 1	11 ± 1

Half-life of pre-mRNA in mouse bone marrow derived macrophages (BMDM) were determined experimentally by ActD treatment under different TNF treatment conditions. The results, represented as time in minutes, are mean ±SD from triplicate samples. "-" refers to those values that cannot be accurately determined because the pre-mRNA levels were too low or fell rapidly beyond the linear range of measurement after treatment of ActD. Location refers to the amplicon location across the 5′ end of an intron (In) if not specifically indicated.

Table S4. Half-life of pre-mRNA in mouse embryonic fibroblasts (3T3)

		Fibroblasts		
Gene	Location	Basal	TNF (30 min)	TNF (6 h)
Housekeeping				
Actb	In 2	4.6 ± 0.2	3.3 ± 0.4	4.4 ± 1.2
Group I				
Cxcl1	In 1	2.7 ± 0.3	2.0 ± 0.1	2.1 ± 0.3
Nfkbia	In 5	3.3 ± 0.7	2.9 ± 0.3	3.2 ± 0.2
Tnfaip3	In 2	5.6 ± 1.5	4.2 ± 0.3	3.2 ± 0.7
Fos	In 1	—	2.9 ± 0.8	4.0 ± 3.7
Group II				
Cxcl10	In 1	—	5.1 ± 1.5	4.3 ± 1.1
Icam1	In 2	—	7.4 ± 6.2	6 ± 4
Nfkbie	In 5	9.0 ± 4.2	13	15
Group III				
Saa3	In 3 (3′)	6.8 ± 1.3	4.2 ± 0.4	3.7 ± 0.2
Mmp3	In 8	—	10 ± 2	8.3 ± 3.2
Ccl5	In 2	12 ± 5	20 ± 12	10.5 ± 3.9
Gbp2	In 1	—	>30	16 ± 3
Ifit1	In 1	—	>30	24 ± 11

For methods and notes, see Table S3.

Other Supporting Information Files

Datasets S1 (XLSX)